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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/810,358	03/26/2004	Ker-Sang Chen	9188R&	1244

27752 7590 05/29/2007

THE PROCTER & GAMBLE COMPANY
INTELLECTUAL PROPERTY DIVISION - WEST BLDG.
WINTON HILL BUSINESS CENTER - BOX 412
6250 CENTER HILL AVENUE
CINCINNATI, OH 45224

EXAMINER
SHAFER, SHULAMITH H

ART UNIT	PAPER NUMBER
1647	

MAIL DATE	DELIVERY MODE
05/29/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/810,358	CHEN ET AL.	
	Examiner	Art Unit	
	Shulamith H. Shafer, Ph.D.	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 February 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-45* is/are pending in the application.

4a) Of the above claim(s) *13-15 and 24-45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12 and 16-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *2/22/07*.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Status of Application, Amendments, And/Or Claims:

The amendment received 22 February 2007 in response to the Office Action of 23 August 2006 has been entered. Upon further consideration, the requirement for election of species A, anti-inflammatory cytokines and B, pro-inflammatory cytokines made in Requirement for Restriction of 16 May 2006 is withdrawn.

Claims 1-45 are pending in the instant application. Claims 24-45 are withdrawn as being drawn to a non-elected invention. Claims 13-15 are withdrawn as being drawn to a non-elected invention.

Claims 1-12, and 16-23 are under consideration.

Withdrawn Objections/Rejections

Objections

The objection to Claims 2-5 is withdrawn in view of withdrawal of requirement for restriction.

The objection to Claim 12 is withdrawn in response to Applicant's argument.

Rejections

The rejection of claims 2 and 3 under 35 U.S.C. 112, second paragraph as reciting improper Markush groups is withdrawn in view of applicant's amendment to the claim.

The rejection of Claims 1, 6 and 22 under 35 U.S.C. 112, second paragraph as reciting the terms "anti-inflammatory cytokine" and "pro-inflammatory cytokine" is withdrawn in view of applicant's argument.

The rejection of Claim 6 under 35 U.S.C. 112, second paragraph as reciting "wherein said ratio of anit-inflammatory cytokine to pro-inflammatory cytokine is

interleukin-10/interleukin-12" is withdrawn in view of applicant's amendment to the claims.

The rejection of Claims 9-11 under 35 U.S.C. 112, second paragraph for reciting "peripheral blood mononuclear cells with in vitro stimulation" is withdrawn in view of applicant's arguments.

The rejection of Claim 12 under 35 U.S.C. 112, second paragraph reciting "wherein said in vitro stimulation comprises a mitogen, probiotic, anti-CD3 molecule" is withdrawn in view of applicant's amendment to the claim.

The rejection of Claim(s) 16-21 under 35 U.S.C. 112, second paragraph for lacking antecedent basis is withdrawn in view of applicant's amendment to the claim.

The rejection of Claim(s) 1-6, 9-12, 16-23 under 35 U.S.C. 112, first paragraph, is withdrawn in view of applicant's arguments. New rejections under 35 U.S.C. 112, first paragraph will be discussed below.

Maintained/New Objections or Rejections

Objections

Information Disclosure:

References submitted on IDS filed 22 February 2007 are not in compliance with the provisions of 37 CFR 1.97, 1.98 and MPEP § 609 for the following reasons: References 5 and 6 on page 1 of 2 do not indicate the source or year; Reference 7 on page 1 of 2 does not indicate source of reference; reference 3 on page 2 of 2 does not indicate the source or year; reference 6 on page 2 of 2 does not indicate the source of the text. Therefore, these references have been lined through and have not been considered. Applicant's attention is directed to MPEP § 609 and 37 CFR 1.98(b) where it states: Each publication listed in an information disclosure statement must be identified by publisher, author (if any), title, relevant pages of the publication, date, and place of publication.

Claims:

Claims 18 and 21 are objected to because of the following informalities: There are grammatical errors in the claims; the claims recite "a flow cytometer detection systems". Appropriate correction is required.

Rejections

35 U.S.C. § 112, Second Paragraph:

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The rejection of Claims 1-12, and 16-23 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is maintained and applied to rejoined claims 7 and 8 for reasons of record and for reasons set forth below.

Claim 1, the independent claim of the instant invention is an incomplete method claim. The method steps recite measuring the level of at least one anti-inflammatory cytokine and at least one pro-inflammatory cytokine in a biological sample before and after treatment. The method taught in the working example comprises drawing venous blood from each subject before and after oral feeding with a probiotic preparation (treatment). PBMCs isolated from blood of patients were incubated *in vitro* with a probiotic preparation. The quantity of cytokines in supernatant of culture media was measured. Therefore, cytokines were not measured in a biological sample from a mammalian subject, as required by the claim; rather, cytokines secreted by cells stimulated *in vitro* were measured in the exemplary method disclosed in the specification. Thus, the claimed method is not the method disclosed in the specification. It is therefore unclear what the claim is directed to.

Claims 2-12 and 16-21 are included in this part of the rejection as dependent upon rejected claim.

Claims 2-5, 9-12, 16, 17, 19 and 20 are vague and indefinite for reciting "mixtures thereof".

Applicant traverses the rejection. The reasons for the traversal are:

a. with respect to claims 2-5: The claims are a proper Markush group. One of skill in the art would understand that levels of one, two or more cytokines could be measured and ratios of levels compared.

b. with respect to claims 9-11: The claims, as amended are proper Markush groups and thus, it is clear that more than one of the sample types may be taken and tested.

c. with respect to claim 12: The claim, as amended is a proper Markush group; thus one would understand that stimulation could occur either one or more members of the recited group.

d. with respect to 16, 17, 19 and 20: The claims, as amended are proper Markush groups; thus, one could measure levels of cytokines using one or more than one method.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

With respect to a: It is unclear how many of the claimed cytokines applicant intends to measure. Furthermore, the inflammatory cytokine cannot be a mixture of two or more cytokines.

With respect to b: It is also unclear if applicant intends all of the recited elements (biological samples) to be used together or separately. Furthermore, a biological sample cannot be a mixture of one or more biological samples.

With respect to c: As stated in the previous Office Action, it is unclear if applicant intends all of the recited elements (compounds which stimulate PBMCs) to be used together or separately. It is unclear if applicant intends to add two or more elements at the same time or sequentially.

With respect to d: It is unclear how one can have mixtures of different assay methods. The term "mixture", as commonly understood refers to a combination of different substances.

Claims 18 and 21 are included in this part of the rejection as dependent upon rejected claims.

Claim 22 remains rejected under 35 U.S.C. 112, 2nd paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant traverses the rejection. The reason for the traversal are:

A system does not necessarily have to contain structural limitations

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

Claim 22 is considered indefinite because a kit, by definition, must contain two or more elements and the interrelationships between the elements must be explicitly stated (see In re Venezia 530 F.2d 956 CCPA 1975).

Claim 23 is included in this part of the rejection as dependent upon a rejected claim.

35 U.S.C. § 112, First Paragraph:

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim(s) 1-12 and 16-21 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. **This is a scope of enablement rejection**.

The specification is enabling for a method of determining the efficacy of a treatment of inflammatory diseases of the bowel in mammals *in vivo*:

a. wherein the cytokine levels are measured before and after treatment in a biological sample wherein the biological sample comprises a biopsy sample from the bowel, peripheral blood mononuclear cells with in vitro stimulation, gut lymphoid tissues with *in vitro* stimulation or gut lymphoid tissues without *in vitro* stimulation

b. wherein a treatment comprises administration of a treatment and/or a composition other than direct administration of anti-inflammatory cytokines or compositions which directly inhibit cytokines

c. wherein the changes in ratios following administration of treatment which are indicative of efficacy of treatment (for inflammatory diseases of the bowel) are changes in ratios of levels of IL-10 to levels of IL-12, levels of IL-10 to levels of TNFα, or levels of IL-10 to levels of IFNγ

The specification does not provide enablement for the full scope of the claims.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention, if not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

The claims are broadly drawn to a method of determining the efficacy of any treatment of inflammatory disease of the bowel comprising the following steps:

(a) measuring the level of at least one anti-inflammatory cytokine and at least one pro-inflammatory cytokine in a biological sample from a mammalian subject;

(b) determining the ratio of the level of the at least one anti-inflammatory cytokine to the at least one pro-inflammatory cytokine;

c) administering any, unspecified, treatment

d) repeating steps (a) and (b)

With respect to biological samples:

The method of the present invention comprises measuring cytokine levels in a biological sample obtained from a mammalian subject both before and during or after administration of treatment. The specification discloses that biological samples include: urine, plasma, serum, saliva, tissue biopsies, cerebrospinal fluid, peripheral blood mononuclear cells (PBMCs) with *in vitro* stimulation, peripheral blood mononuclear cells without *in vitro* stimulation, gut lymphoid tissues with *in vitro* stimulation, gut lymphoid tissues without *in vitro* stimulation, and gut lavage fluids [paragraph 0059 PGPUB 20040228837, the PGPUB of the instant application].

Cytokine levels in urine, plasma, and serum represent the sum total of cytokines from all tissues and organs; cytokine levels in saliva, tissue biopsies, or cerebral spinal fluid are indicative of cytokine levels in specific, localized tissues. Thus, changes of cytokine levels in these samples may not be indicative of changes in the pathology at the sites of tissues effected by IBD.

The art teaches: Whiteside (2003. Chapter 61 in The Cytokine Handbook, Vol II, 4th edition, pages 1384-1386 enclosed) teach measurements of plasma levels of cytokines are often not meaningful. In lieu of plasma assays, spontaneous or stimulated production of cytokines by PBMC can be evaluated. The former is a measure of in vivo activation of these cells, while the latter can be viewed as a reliable measure of immune competence. The assay of spontaneous production of cytokines by PBMC is based on the rationale that immune cells activated *in vivo* will spontaneously produce cytokines. However, any in vitro manipulation of cells prior to the assay will tend to give false-positive results.

The working example teaches:

Daily oral feeding with probiotic preparation for 3 weeks increased the ratio IL-10/IL-12, IL-10/TNF-α and/or IL-10/IFN-γ in IBS patients' PBMC upon pro-biotic *in vitro* stimulation [paragraph 0083, 0084]. Additionally, mean abdominal pain/discomfort decreased in treated IBS patients [paragraph 0084]. The disclosure teaches "The negative correlation between the change in abdominal pain/discomfort and the change in IL-10 to IL-12 ratio indicated that the increase in IL-10 to IL-12 ratio was associated with the relief from IBS symptom of abdominal pain/discomfort" [paragraph 0085].

Thus, only methods which measure cytokine levels in tissues directly from the bowel region or methods which measure cytokine production by peripheral blood mononuclear cells with *in vitro* stimulation, gut lymphoid tissues with *in vitro* stimulation or gut lymphoid tissues without *in vitro* stimulation are enabled.

With respect to treatment:

The specification teaches that the treatments may be any treatment and/or composition for use in the treatment of inflammatory diseases of the bowel (IBS) [paragraph 0056]. Thus, the specification envisions using the methods of the instant invention to determine the efficacy of any treatment for IBS including such art-recognized treatments as direct administration of cytokines and anti-cytokines.

The art teaches that IB diseases are controlled by a wide variety of treatments including dietary regimens, and drugs including antirheumatic drugs and immunomodulators. Some treatment modalities involve administration of cytokines and anti-cytokines, including anti-TNF-α and IL-10 (Papadikis et al, page 295, 4th paragraph cited on previous IDS). Thus, patients may be treated by administration of IL-10 and/or antibodies to pro-inflammatory cytokines. Direct administration of IL-10 and/or antibodies which bind to pro-inflammatory cytokines would result in raising the levels of IL-10 in a biological sample and/or decreasing the levels of pro-inflammatory cytokines. This increase in IL-10 levels and/or decrease in pro-inflammatory cytokines would naturally result in a shift in the ratio of levels IL-10 to levels of IL-12 or ratio of levels IL-10 to levels of TNF-α, or ratio of levels IL-10 to levels of IFNγ but these changes would

not be indicative of efficacy of treatment. These changes would arise as a direct result of administration of IL-10 and/or antibodies which bind to pro-inflammatory cytokines.

Applicant traverses this part of the rejection (Response of 22 February 2007, page 18, 2nd paragraph). Applicant states "the Examiner confusingly then states that the alterations in cytokines ratios would be due to administration of the drugs and would not indicate efficacy of treatment".

Examiner apologizes for any confusion. As stated above, administration of IL-10 or antibodies to pro-inflammatory cytokines are art-recognized methods of treating IBD. Thus, this treatment itself would result in increasing IL-10 levels and/or decreasing pro-inflammatory cytokine levels in biological samples drawn from the patient. These changes in cytokine levels would result in a change in the ratios of levels IL-10 to levels of IL-12 or levels of TNF-α or levels of IFN-γ, but would not be indicative of efficacy of treatment.

Therefore, applicant is not enabled for treatment that involves administering anti-inflammatory cytokines or compositions which interact directly with pro-inflammatory cytokines.

With respect to changes in ratios of the levels of anti-inflammatory cytokines to pro-inflammatory cytokines following administration of treatment as indicative of efficacy of treatment:

The specification teaches that the "methods of the present invention comprise the step of measuring at least one anti-inflammatory and at least one pro-inflammatory cytokine levels in a biological sample obtained from a mammalian subject (page 11, lines 4-6).

Rogler et al (1998. World J Surg. 22:382-389, cited on IDS of 20 July 2004, page 4 of 5, reference 8) teach that cytokines play a central role in modulation of the intestinal immune system and lists numerous cytokines having pro- and anti-inflammatory functions (abstract). However, the working examples recite only one anti-inflammatory cytokine, IL-10, and three pro-inflammatory cytokines, IL-12, TNF-α or IFN-γ as

cytokines measured and recite only ratios of IL-10/IL-12, IL-10/TNF-α and IL-10/IFN- γ as the ones evaluated in the methods of the instant invention.

The specification additionally teaches "it is believed that the specific ratios described herein (IL-10/IL-12, IL-10/TNF-α and IL-10/IFN-γ) are pivotal to the progression or remission of inflammatory diseases of the bowel, and therefore alterations in the ratios herein are indicative of the inhibition or promotion of disease effects by treatments being investigated....." [0076].

Thus, one of ordinary skill in the art would be unable to predict that changes in the ratio of the levels of any anti-inflammatory cytokine to the levels of any pro-inflammatory cytokine following treatment would indicate the efficacy of a treatment for inflammatory bowel disease. Therefore, only methods which determine changes in the ratio of the levels of IL-10/IL-12, IL-10/TNF-α and IL-10/IFN-γ after treatment as indicative of efficacy of treatment are enabled.

Applicants' claims are excessively broad due to the diverse treatment modalities encompassed, and the unpredictability that a change in ratios of level of any anti-inflammatory cytokines to level of any pro-inflammatory cytokines in any biological sample would be indicative of efficacy of treatment.

Due to the large quantity of experimentation necessary to determine that changes in any cytokine levels in any biological sample would be indicative of an efficacy of treatment, the lack of direction/guidance presented in the specification regarding the same, the absence of working examples directed to the same, the complex nature of the invention, the state of the prior art which establishes that some treatments of IBD comprise direct administration of cytokines, and that assays of cytokine levels in some biological samples are often not meaningful, and the breadth of the claims which encompass any treatment modality and assays utilizing any biological sample, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention.

35 U.S.C. § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The rejection of Claim 22 under 35 U.S.C. 102(b) as being anticipated by Vignali (2000 Journal of Immunological Methods 243:243-255) is maintained for reasons of record and for reasons set forth below.

Applicant traverses the rejection. The reasons for the traversal are:

a. While Vignali discloses multiple cytokine levels can be measured simultaneously, the reference discloses nothing with respect to ratios of cytokines or using said ratios to indicate anything

b. Vignali does not disclose a kit.

c. Vignali does not disclose providing instructions to users of a kit.

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

Claim 22, given its broadest reasonable interpretation, requires a product for measuring multiple cytokines in a biological sample from a mammalian subject. There are no structural limitations recited as to the contents of said kit.

With respect to a: Determination of ratios and using said ratios to indicate something are computational and/or mental steps. Such steps do not convey patentability. Vignali teaches a FlowMetrix System of quantifying the concentration of a number of cytokines simultaneously in a 100 µl sample. Once the concentration of cytokines are determined, the ratios may easily be determined by dividing the concentrations of anti-inflammatory cytokines by the concentrations of pro-inflammatory cytokines. The skilled artisan may then draw conclusions by comparing ratios.

With respect to b: Vignali teaches use of a FlowMetrix System. This system is sold by Invitrogen as Protein Multiplex Immunoassays. The company states "Biosource

now offers over 100 kits for uses with the Luminex xMAP system" (see, for teaching purposes only, http://www.invitrogen.com/content.cfm?pageid+11317&CID=KNC-GOOGLE&s_kwcid=..., downloaded 20 May 2007). Furthermore, there are no structural limitations recited in the claims as to the contents of said kit. A reasonable interpretation of the claims could interpret a kit to comprise, for example, antibodies to cytokines in a vial or test tube.

With respect to c: Where the only difference between a prior art product (Multiplex system) and a claimed product (kit) is printed matter that is not functionally related to the product, the content of the printed matter will not distinguish the claimed product from the prior art. In re Ngai, 367 F.3d 1336, 1339, 70 USPQ2d 1862, 1864 (Fed. Cir. 2004) (Claim at issue was a kit requiring instructions and a buffer agent. The Federal Circuit held that the claim was anticipated by a prior art reference that taught a kit that included instructions and a buffer agent, even though the content of the instructions differed.). See also In re Gulack, 703 F.2d 1381, 1385-86, 217 USPQ 401, 404 (Fed. Cir. 1983)("Where the printed matter is not functionally related to the substrate, the printed matter will not distinguish the invention from the prior art in terms of patentability .... [T]he critical question is whether there exists any new and unobvious functional relationship between the printed matter and the substrate"). See MPEP 2112.01, Section III.

35 U.S.C. § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The rejection of Claim 23 under 35 U.S.C. 103(a) as being unpatentable over Vignali (2000 Journal of Immunological Methods 243:243-255) is maintained for reasons of record and reasons set forth below.

Applicant traverses the rejection. The reasons for the traversal are:

a. Vignali does not disclose a kit

b. The reference discloses nothing with respect to ratios of cytokines or using said ratios to indicate anything

c. Vignali does not disclose providing instructions to users of a kit.

d. There is no motivation to add a means for collecting biological samples

Applicant's arguments have been fully considered but are not found to be persuasive for the following reasons:

With respect to a, b, and c: See discussion above

With respect to d: Vignali et al teaches measurement of a number of cytokines in a single biological sample. One of ordinary skill in the art would understand that the biological sample must be obtained from the mammalian subject. Therefore, one of ordinary skill in the art would be motivated to include an instrument or sampling device to obtain said biological sample to increase efficiency of utilization of assay to determine cytokine levels.

Claims 1-5, 9, 16, 17, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. (2002 Am J. Physiol, Gastrointestinal Liver Physiol 283:G187-G195). Togawa et al. teach administration of lactoferrin to rats; colonic inflammation was induced in experimental animals by intracolonic injection of TNBS (controls received water intracolonically in place of TNBS) (abstract and page G187, 2nd column, last paragraph, G188, 1st column, 3rd paragraph). The reference teaches measurement of anti- and pro-inflammatory cytokines in samples of inflamed colon seven days after TNBS administration (page G188, 2nd column, 2nd paragraph, last paragraph bridging G189, 1st column, 1st paragraph, page G191, 1st column, last paragraph bridging 2nd column, first paragraph and Figure 5) and in samples of control colons. The reference teaches measurement of the pro-inflammatory cytokines TNF-α,

and IL-1β, and the anti-inflammatory cytokines IL-4, and IL-10 by ELISA assays (abstract and G189, 1st column, 1st paragraph) and comparing cytokine levels in treated rats to those in untreated rats (Figure 5). Togawa et al does not teach measuring the level of anti-inflammatory and pro-inflammatory cytokines before administering treatment, measuring levels in tissue biopsies, or determining the ratio of levels of anti-inflammatory cytokine to level of pro-inflammatory cytokine.

However, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to measure cytokine levels in a biological sample (tissue biopsy) before administration of treatment and after treatment. A person of ordinary skill in the art would have been motivated to make those modifications because Togawa et al teach "although it is not possible to extrapolate findings from animal models to the clinical situation, these data suggest that lactoferrin is potentially attractive as a therapeutic strategy for the treatment of inflammatory bowel disease" (page G194, 1st column, 1st paragraph), thus suggesting clinical experimentation to determine efficacy of the described therapeutic approach. Thus, the skilled artisan, following the teaching of Togawa et al, would be motivated to measure cytokine levels before treatment in a clinical setting, instead of measuring levels in control animals and would be motivated to measure cytokine levels in biological samples such as tissue biopsies, instead of measuring levels in tissue removed from the control and experimental animal. Furthermore, knowing the results of measurements of cytokine levels (as shown, for example, in Figure 5), one would be motivated to compute ratios as a way of determining shifts in patterns of cytokine levels. One would reasonably expect success because method of measuring cytokine levels in biological samples is well known in the art, and is taught by Togawa et al.

Claims 18 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. as applied to claims 1, 17 and 20 in view of Vignali et al. (cited above and in previous Office Action). The teachings of Togawa et al. are outlined above. Togawa et al. does not teach a method of measuring levels of at least one anti-inflammatory cytokine and at leas one pro-inflammatory cytokine in a biological sample

by multiplexed ELISAs using coded microspheres coupled with a flow cytometer detection system. Vignali teaches a FlowMetrix System of quantifying the concentration of 15 cytokines simultaneously in a 100 µl sample. The Luminex FlowMetrix system uses microspheres as the solid support for a conventional immunosorbent assay. Each bead set is comprised of microspheres manufactured with a uniform, distinct proportion of red and orange fluorescent dyes. Data are acquired on a conventional flow cytometer (page 246, 1st column, 1st paragraph).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and substitute the multiplex assay taught by Vignali for the ELISA assay taught by Togawa et al. The person of ordinary skill in the art would have been motivated to make these modification because Vignali teaches the multiplex system simultaneously measures many different analytes in a small sample volume (abstract). One would have expected success because Vignali teaches utilizing the multiplex assay to measure a number of cytokines simultaneously in biological fluids and tissue culture samples.

Claims 6-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Togawa et al. as applied to claim 1 in view of Blumberg et al. (1999. Current Opinion in Immunology 11:648-656). The teachings of Togawa et al. are outlined above. Togawa et al do not teach method wherein the ratio of anti-inflammatory cytokine to pro-inflammatory cytokine is the ratio of the level of IL-10/to the level of IL-12 (claim 6), or the level of TGF-β/to the level of IL-12 (claim 7), or the level of IL-10/ to the level of IFN-γ (claim 8). Togawa et al. teaches measurement of anti-inflammatory cytokines IL-4 and **IL-10** and measurement of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (abstract and Figure 5). Blumberg et al. teach immune responses uniquely involved in IBD pathogenesis and note the importance of balance of pro-inflammatory cytokines such as **IFN-γ, TNF**, and **IL-12** and anti-inflammatory cytokines such as **IL-10** and **TGF-β** (abstract). The reference teaches that IL-12 is a key factor in the pathogenesis of the TNBS-induced colitis model (the model taught by Togawa et al) and induces overproduction of IFN-γ and TNF (page 650, 2nd column, last paragraph bridging page

651, 1st column, 3rd paragraph). Blumberg et al also teach that mucosal inflammation can be viewed as a failure of production of suppressor cytokines such as TGF-β and IL-10 (page 652, 2nd column, 2nd paragraph).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the teachings of Togawa et al and substitute measurement of the pro-inflammatory cytokines taught by Blumberg et al (IFN-γ and IL-12) for the pro-inflammatory cytokine taught by Togawa et al (TNF-α, IL-1β and IL-6) and the anti-inflammatory cytokine taught by Blumberg et al (TGF-β) for the anti-inflammatory cytokine taught by Togawa et al (IL-10). Once measurement of these cytokines is accomplished, the calculation of ratios would be obvious as a way of monitoring changes in the balance of levels of pro- to anti- inflammatory cytokines. One would be motivated to make these modifications because both references teach disturbed balance between proinflammatory and anti-inflammatory cytokines in inflammatory bowel disease and Blumberg et al teach IFN-γ, TNF, and IL-12 are pro-inflammatory cytokines involved in pathology of IBD and IL-10 and TGF-β are anti-inflammatory cytokines whose expression may be down-regulated in IBD. One would have expected success because methods of measuring cytokine levels in biological samples is well known in the art, and is taught by Togawa et al.

Conclusions:

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shulamith H. Shafer, Ph.D. whose telephone number is 571-272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol, Ph.D. can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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SHS

LORRAINE SPECTOR
PRIMARY EXAMINER